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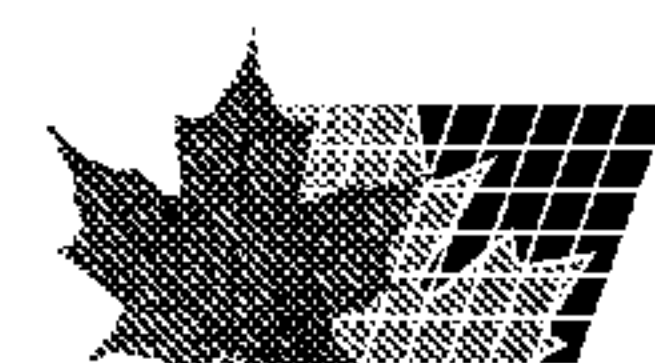
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(57) **Abrégé/Abstract:**

The present invention features polypeptides and nucleic acids related to a dog MCH receptor and uses of such polypeptides and nucleic acids. The dog MCH receptor is a G protein coupled receptor whose activity is stimulated by MCH binding.



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(54) Title: DOG MELANIN-CONCENTRATING HORMONE RECEPTOR

(57) Abstract: The present invention features polypeptides and nucleic acids related to a dog MCH receptor and uses of such polypeptides and nucleic acids. The dog MCH receptor is a G protein coupled receptor whose activity is stimulated by MCH binding.

TITLE OF THE INVENTION

DOG MELANIN-CONCENTRATING HORMONE RECEPTOR

CROSS-REFERENCE TO RELATED APPLICATIONS

- 5 The present application claims priority to provisional application U.S. Serial No. 60/219,669, filed July 21, 2000, hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

- 10 The references cited herein are not admitted to be prior art to the claimed invention.

- Neuropeptides present in the hypothalamus play a major role in mediating the control of body weight. (Flier *et al.*, 1998, *Cell*, 92, 437-440.) Melanin-concentrating hormone (MCH) is a cyclic 19-amino acid neuropeptide synthesized as part of a larger pre-prohormone precursor in the hypothalamus that also
15 encodes neuropeptides NEI and NGE. (Nahon *et al.*, 1990, *Mol. Endocrinol.*, 4, 632-637.) MCH was first identified in salmon pituitary, and in fish MCH affects melanin aggregation thus affecting skin pigmentation. In trout and in eels MCH has also been shown to be involved in stress induced or CRF-stimulated ACTH release. (Kawauchi *et al.*, 1983, *Nature*, 305, 321-323.)

- 20 In humans two genes encoding MCH have been identified that are expressed in the brain. (Breton *et al.*, 1993, *Mol. Brain Res.*, 18, 297-310.) In mammals MCH has been localized primarily to neuronal cell bodies of the hypothalamus which are implicated in the control of food intake, including perikarya of the lateral hypothalamus and zona inertia. (Knigge *et al.*, 1996, *Peptides*, 17, 1063-
25 1073.)

- Pharmacological and genetic evidence suggest that the primary mode of MCH action is to promote feeding (orexigenic). MCH mRNA is up regulated in fasted mice and rats and in the *ob/ob* mouse. (Qu *et al.*, 1996, *Nature*, 380, 243-247.) Injection of MCH centrally (ICV) stimulates food intake and MCH antagonizes the
30 hypophagic effects seen with α -melanocyte stimulating hormone (α MSH). (Qu *et al.*, 1996, *Nature*, 380, 243-247.) MCH-deficient mice are lean, hypophagic, and have increased metabolic rate. (Shimada *et al.*, 1998, *Nature*, 396, 670-673.)

- MCH action is not limited to modulation of food intake as effects on the hypothalamic-pituitary-axis have been reported. (Nahon, 1994, *Critical Rev. in*
35 *Neurobiol.*, 8, 221-262.) MCH may be involved in the body response to stress as

MCH can modulate the stress-induced release of CRF from the hypothalamus and ACTH from the pituitary. In addition, MCH neuronal systems may be involved in reproductive or maternal function.

Several references describe a receptor that is indicated to bind MCH.
5 (Chambers *et al.*, 1999, *Nature*, 400, 261-265; Saito *et al.*, 1999, *Nature*, 400, 265-269; Bächner *et al.*, 1999, *FEBS Letters*, 457, 522-524; Shimomura *et al.*, 1999, *Biochemical and Biophysical Research Communications*, 261, 622-626; and Lembo *et al.*, 1999, *Nat. Cell Biol.*, 1, 267-271.)

10 SUMMARY OF THE INVENTION

The present invention features polypeptides and nucleic acids related to a dog MCH receptor and uses of such polypeptides and nucleic acids. The dog MCH receptor is a G protein coupled receptor whose activity is stimulated by MCH binding.

Polypeptides related to a dog MCH receptor contain a region of at least
15 9 contiguous amino acids that is present in a dog MCH receptor. Such polypeptides may contain additional regions including regions present, or not present, in a dog MCH receptor.

Nucleic acids related to a dog MCH receptor contain a region of at least 18 contiguous nucleotides that is present in a dog MCH receptor nucleic acid.
20 Such nucleic acids may contain additional regions including regions present, or not present, in a dog MCH receptor nucleic acid.

Thus, a first aspect of the present invention describes a purified polypeptide comprising a unique amino acid region of a dog MCH receptor. The unique region is at least 9 amino acids in length.

25 A "unique amino acid region" of a dog MCH receptor is a region of contiguous amino acids present in SEQ. ID. NO. 1 that is not present in SEQ. ID. NOs. 2 or 3. SEQ. ID. NO. 1, which is referred to herein as a dog MCH receptor, was derived from dog nucleic acid using a human primer and may thus contain one or more N-terminal amino acids corresponding to a human source rather than a dog
30 source. SEQ. ID. NO. 2 is a human MCH receptor amino acid sequence and SEQ. ID. NO. 3 is a rat MCH receptor amino acid sequence. The unique region may contain segments of contiguous amino acids present in SEQ. ID. NOs. 2 or 3 smaller than the indicated unique region size.

A "purified polypeptide" represents at least 10% of the total protein
35 present in a sample or preparation. In preferred embodiments, the purified

polypeptide represents at least about 50%, at least about 75%, or at least about 95% of the total protein in a sample or preparation. Reference to “purified polypeptide” does not require that the polypeptide has undergone any purification and may include, for example, chemically synthesized polypeptide that has not been purified.

5 Another aspect of the present invention describes a purified nucleic acid comprising a nucleotide sequence encoding for a unique amino acid region from a dog MCH receptor. The encoded for region is at least 9 amino acids in length.

 A “purified nucleic acid” represents at least 10% of the total nucleic acid present in a sample or preparation. In preferred embodiments, the purified
10 nucleic acid represents at least about 50%, at least about 75%, or at least about 95% of the total nucleic acid in a sample or preparation. Reference to “purified nucleic acid” does not require that the nucleic acid has undergone any purification and may include, for example, chemically synthesized nucleic acid that has not been purified.

 Another aspect of the present invention describes a purified nucleic
15 acid comprising a unique nucleotide sequence region of a dog MCH receptor nucleic acid sequence, or the complement thereof. The unique nucleotide sequence region is at least 18 nucleotides in length.

 A “unique nucleotide sequence region” of a dog MCH receptor nucleic acid is a region that comprises at least 18 contiguous nucleotides of SEQ. ID. NO. 4
20 that is not present in SEQ. ID. NOs. 5 or 6. SEQ. ID. NO. 4, which is referred to herein as a nucleotide sequence encoding for a dog MCH receptor, was derived from dog nucleic acid using a human primer and may thus contain one or more 5’ nucleotides corresponding to a human source rather than a dog source. SEQ. ID. NO. 5 is the nucleotide sequence encoding for a human MCH receptor and SEQ. ID. NO. 6
25 is the nucleotide sequence encoding for a rat MCH receptor. The unique region may contain segments of contiguous nucleotides present in SEQ. ID. NOs. 5 or 6 smaller than the indicated unique region size.

 Another aspect of the present invention describes a nucleic acid comprising a recombinant nucleotide sequence encoding for a unique amino acid
30 region of a dog MCH receptor. In different embodiments the nucleic acid is an expression vector or is part of a host genome.

 A “recombinant nucleotide sequence” is a sequence that is present on a nucleic acid containing one or more nucleic acid regions not naturally associated with that sequence. Examples of such regions that may be present with the sequence

include one or more regulatory elements not naturally associated with the sequence, viral elements, and selectable markers.

Another aspect of the present invention describes a recombinant cell comprising an expression vector encoding for a unique amino acid region of a dog
5 MCH receptor. The expression vector contains a promoter that is functionally coupled to nucleic acid encoding for the unique region and is recognized by an RNA polymerase present in the cell.

Another aspect of the present invention describes a recombinant cell made by introducing an expression vector encoding for a unique amino acid region of
10 a dog MCH receptor into a cell. The expression vector can be used to insert the dog nucleic acid into the genome of the host, or can exist as an autonomous piece of nucleic acid.

Another aspect of the present invention describes a method of measuring the ability of a test compound to affect MCH receptor activity. The
15 method involves providing the compound to a recombinant cell expressing a functional MCH receptor containing a unique dog amino acid region from a recombinant nucleic acid and measuring MCH receptor activity. Preferably, the recombinant nucleic acid is present on an expression vector.

Another aspect of the present invention describes a method of
20 producing a MCH receptor polypeptide. The method involves the step of growing a recombinant cell able to express a dog MCH receptor polypeptide under conditions wherein the polypeptide is expressed from an expression vector.

Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples.
25 The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

30 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a comparison of the amino acid sequence for a dog MCH receptor (SEQ. ID. NO. 1), a human MCH receptor (SEQ. ID. NO. 2), and a rat MCH receptor (SEQ. ID. NO. 3).

Figures 2A-2C illustrate a comparison of the nucleotide sequence encoding for a dog MCH receptor (SEQ. ID. NO. 4), a human MCH receptor (SEQ. ID. NO. 5), and a rat MCH receptor (SEQ. ID. NO. 6).

5 DETAILED DESCRIPTION OF THE INVENTION

Polypeptides and nucleic acids related to a dog MCH receptor are preferably used in an *in vitro* functional assay measuring whether a compound acts differently at the dog receptor than at the human receptor, or affects MCH receptor activity. Such assays can be used to help evaluate whether a dog model provides a
10 useful test system in looking for a human therapeutic compound and for assaying for compounds active at the MCH receptor.

The MCH receptor provides a target to achieve different beneficial effects in a patient. Preferably, MCH receptor activity is modulated to achieve one or more of the following: weight loss, weight gain, treat cancer (*e.g.*, colon or breast),
15 reduce pain, treat diabetes, reduce stress, or treat sexual dysfunction.

Modulation of MCH receptor activity can be achieved by evoking a response at the MCH receptor or by altering a response evoked by an MCH receptor agonist or antagonist. Compounds modulating MCH receptor activity include agonists, antagonists, and allosteric modulators. Generally, MCH receptor antagonists
20 and allosteric modulators negatively affecting activity will be used to achieve weight loss, treat cancer (*e.g.*, colon or breast), reduce pain, reduce stress, and/or treat sexual dysfunction; and MCH receptor agonists and allosteric modulators positively affecting activity will be used to produce a weight gain.

Preferably, MCH receptor activity is modulated to achieve a weight
25 loss or to treat diabetes in a patient. Diabetes mellitus can be treated by modulating MCH receptor activity to achieve, for example, one or both of the following: enhancing glucose tolerance or decreasing insulin resistance.

Excessive body weight is a contributing factor to different diseases, including hypertension, diabetes, dyslipidemias, cardiovascular disease, gall stones,
30 osteoarthritis, and certain forms of cancers. Bringing about a weight loss can be used, for example, to reduce the likelihood of such diseases and as part of a treatment for such diseases. Weight reduction can be achieved by modulating MCH receptor activity to obtain, for example, one or more of the following effects: reducing appetite, increasing metabolic rate, reducing fat intake, or reducing carbohydrate
35 craving.

Facilitating a weight gain, maintenance in weight, or appetite increase is particularly useful for a patient having a disease or disorder, or under going a treatment, accompanied by weight loss. Examples of diseases or disorders accompanied by weight loss include anorexia, bulimia, cancer cachexia, AIDS, wasting, cachexia, and wasting in frail elderly. Examples of treatments accompanied by weight loss include chemotherapy, radiation therapy, temporary or permanent immobilization, and dialysis.

MCH RECEPTOR RELATED POLYPEPTIDES

Polypeptides related to the dog MCH receptor preferably contain a unique dog amino acid region. In addition to the unique amino acid region, regions that may, or may not, be related to the dog MCH receptor polypeptide may be present. Such polypeptides have a variety of uses, such as providing a component of a functional MCH receptor; being used as an immunogen to produce antibodies binding to the MCH receptor; being used as a target to identify compounds binding to the MCH receptor; and/or being used in assays measuring the ability of a compound to affect MCH receptor activity.

Unique dog amino acid regions can readily be identified based on a comparison of the dog MCH receptor sequence described herein, with the human and rat MCH receptor amino acid sequences. Such a sequence comparison is illustrated in Figure 1. Examples of unique dog amino acid regions include the following:

SEQ. ID. NO. 7	LEASLLPPGP,
SEQ. ID. NO. 8	SEGPLNLTSGP,
SEQ. ID. NO. 9	RRTGNVSYIN,
SEQ. ID. NO. 10	PFPGGTVGCG, and
SEQ. ID. NO. 11	ILQRMMSVA.

The definition of unique amino acid region is with respect to the human and rat MCH receptors. Thus, a unique amino acid region may be present in a MCH receptor amino acid sequence from one or more species other than the human or rat sequences, or in a non-MCH receptor sequence.

In different embodiments a dog MCH receptor related polypeptide comprises or consists of a unique amino acid region at least 18, at least 27, or at least 54, amino acids in length. Preferably, the dog MCH receptor related polypeptide comprises or consists of the amino acid sequence of SEQ. ID. NO. 1.

Polypeptides can be produced using standard techniques including those involving chemical synthesis and those involving biochemical synthesis. Techniques for chemical synthesis of polypeptides are well known in the art. (See *e.g.*, Vincent, in *Peptide and Protein Drug Delivery*, New York, N.Y., Dekker, 1990.)

5 Biochemical synthesis techniques for polypeptides are also well known in the art. Such techniques employ a nucleic acid template for polypeptide synthesis. The genetic code providing the sequences of nucleic acid triplets coding for particular amino acids is well known in the art. (See, *e.g.*, Lewin *GENES IV*, p. 119, Oxford University Press, 1990.) Examples of techniques for introducing nucleic acid into a
10 cell and expressing the nucleic acid to produce protein are provided in references such as Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, and Sambrook *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

15 Functional MCH Receptor Derivatives

Functional MCH receptors are stimulated by MCH binding. Functional MCH receptors include the MCH receptor of SEQ. ID. NO. 1, and receptors having MCH receptor activity and containing a unique dog amino acid region as a component.

20 Starting with a MCH receptor obtained from a particular source, derivatives can be produced having MCH receptor activity. Such derivatives include polypeptides with amino acid substitutions, additions and deletions. Changes made to produce functional derivatives should be made outside of the MCH binding domain and in a manner not altering the tertiary structure. The ability of a polypeptide to have
25 MCH receptor activity can be confirmed using techniques such as those measuring G-protein activity.

The sequence comparison provided in Figure 1 illustrates amino acids that vary between the human, rat, and dog MCH receptor. Such variable amino acids are good targets for alterations.

30 Additionally, amino acids are classified into certain types based on the structure of their R-groups. Substituting different amino acids within a particular group, such as substituting valine for leucine, arginine for lysine, and asparagine for glutamine may not cause a change in functionality of the polypeptide.

MCH Antibodies

Antibodies recognizing a dog MCH receptor polypeptide can be produced using a polypeptide of SEQ. ID. NO. 1 or a fragment thereof as an immunogen. Fragments should be at least 9 amino acids in length and preferably
5 contain a unique amino acid region.

Antibodies to the MCH receptor have different uses such as being used to identify the presence of MCH receptor polypeptides and for isolating MCH receptor polypeptides. Examples of techniques for producing and using antibodies are described in Ausubel *Current Protocols in Molecular Biology*, John Wiley, 1987-
10 1998, Harlow *et al.*, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988, and Kohler *et al.*, 1975, *Nature*, 256, 495-497.

Binding Assays

Assays measuring the ability of a compound to bind the dog MCH
15 receptor can be performed using a polypeptide of SEQ. ID. NO.1 or a fragment thereof as a target. Fragments should be at least 9 amino acids in length and contain a site to which either an agonist, antagonist, or allosteric modulator binds. Different types of assay formats can be employed including competitive and non-competitive assays.

20 Compounds identified as binding to a full-length receptor or a receptor fragment can be used to determine the locus of a binding site by testing the ability of the compound to bind to smaller length fragments. For example, MCH binds to the MCH receptor and labeled MCH can be used to identify that portion of the receptor to which MCH binds. Fragments identified as containing a compound binding site can
25 be used to test for additional compounds that bind to the binding site.

Preferred polypeptide fragments used in a binding assay consist of a unique amino acid region. However, fragments containing additional amino acid sequences can be employed, for example, to facilitate attachment to a column.

Binding assays can be performed using individual compounds or
30 preparations containing different compounds. A preparation containing different compounds wherein one or more compounds bind to the MCH receptor can be divided into smaller groups to identify compound(s) binding to the MCH receptor. In an embodiment of the present invention a test preparation containing at least 10 compounds is used in a binding assay.

Binding assays can be performed using recombinantly produced MCH receptor polypeptides present in different environments. Such environments include, for example, cell extracts and purified cell extracts containing the MCH receptor polypeptide expressed from recombinant nucleic acid; and the use of a purified MCH receptor polypeptide produced by recombinant means which is introduced into a different environment.

Functional Assays

Assays involving functional dog MCH receptors and chimeric receptors can be employed to select for compounds active at the MCH receptor and to evaluate the ability of a compound to affect receptor activity. MCH receptor activity can be measured using different techniques such as detecting a change in the intracellular conformation of the MCH receptor, measuring G protein activity, or measuring the level of intracellular messengers.

Recombinantly expressed MCH receptor polypeptides can be used to facilitate determining whether a compound is active at the MCH receptor or another receptor. For example, the MCH receptor can be expressed by an expression vector in a cell line such as HEK 293, COS 7, and CHO not normally expressing the receptor, wherein the same cell line without the expression vector or with an expression vector not encoding a MCH receptor can act as a control.

MCH receptor activity can be measured, for example, by assays measuring the phospholipase C signal transduction pathway. Activity of the phospholipase C signal transduction pathway can be measured using standard techniques such as those measuring intracellular Ca^{2+} . Examples of techniques well known in the art that can be employed to measure Ca^{2+} include the use of dyes such as Fura-2 and the use of Ca^{2+} -bioluminescent sensitive reporter proteins such as aequorin. An example of a cell line employing aequorin to measure G protein activity is HEK293/aeq17. (Button *et al.*, 1993, *Cell Calcium*, 14, 663-671, and Feighner *et al.*, 1999, *Science*, 284, 2184-2188, both of which are hereby incorporated by reference herein.)

Chimeric receptors containing one or more MCH receptor regions functionally coupled to polypeptides from other G protein can also be used to measure activity. A chimeric MCH receptor contains an N-terminal extracellular domain; a transmembrane domain made up of transmembrane regions, extracellular loop regions, and intracellular loop regions; and an intracellular carboxy terminus domain.

Preferred chimerics contain one or more of these different domains from a dog MCH receptor.

The specificity of G protein coupling is determined by intracellular domain(s). A chimeric G protein coupled receptor can be produced to functionally couple to a particular G protein. For example a G protein that normally couples to Gs can be coupled to Gq or Gi allowing for the detection of receptor activity by measuring Gq or Gi activity. Techniques for producing chimeric receptors and measuring G protein coupled responses are provided for in, for example, International Application No. WO 97/05252, and U.S. Patent No. 5,264,565, both of which are hereby incorporated by reference herein.

Functional assays can be performed using individual compounds or preparations containing different compounds. A preparation containing different compounds where one or more compounds affect MCH receptor or chimeric receptor activity can be divided into smaller groups of compounds to identify the compound(s) affecting MCH receptor activity. In an embodiment of the present invention a test preparation containing at least 10 compounds is used in a functional assay.

Functional assays can be performed using recombinantly produced MCH receptor polypeptides or chimeric receptor polypeptides present in different environments. Such environments include, for example, cell extracts, and purified cell extracts, containing the MCH receptor polypeptide expressed from recombinant nucleic acid; and the use of a purified MCH receptor polypeptide produced by recombinant means that is introduced into a different environment.

MCH RECEPTOR RELATED NUCLEIC ACID

Nucleic acids related to the dog MCH receptor nucleic acid preferably contain a unique dog nucleotide sequence region or the complement thereof. Such nucleic acids have a variety of uses, such as being used as a hybridization probe or PCR primer to identify the presence of dog MCH nucleic acid; being used as a hybridization probe or PCR primer to identify or clone nucleic acid encoding for receptors related to the MCH receptor from different sources; and/or being used for recombinant expression of a dog MCH receptor polypeptide.

Unique dog nucleic acid regions can readily be identified based on a comparison of the dog MCH receptor nucleic acid sequences described herein, with the human and the rat MCH receptor nucleic acid sequences. Such a sequence comparison is illustrated in Figures 2A-2C.

Examples of unique dog nucleic acid regions include the following:

- SEQ. ID. NO. 12 CCTCGGAGGGCCCGGACAACC,
 SEQ. ID. NO. 13 ACCTCTGCCGGGCCCACCTCGT,
 SEQ. ID. NO. 14 GCCCTTCGTGGTCATCACAGCCGCGTAT,
 5 SEQ. ID. NO. 15 TGTCCTCGGTAGCCCCTGCCTCTCAA, and
 SEQ. ID. NO. 16 TTCGAGCCGTCAGCAATGCT.

The guidance provided in the present application can be used to obtain the nucleic acid sequence encoding the full-length dog MCH receptor, to obtain nucleic acids encoding for MCH receptors from additional sources, and to artificially
 10 produce a MCH receptor. Obtaining nucleic acids encoding a MCH receptor from different sources is facilitated using sets of degenerative probes and primers and by the proper selection of hybridization conditions. Sets of degenerative probes and primers are produced taking into account the degeneracy of the genetic code. Adjusting hybridization conditions is useful for controlling probe or primer specificity
 15 to allow for hybridization to nucleic acids having similar sequences.

Techniques employed for hybridization detection and PCR cloning are well known in the art. Nucleic acid detection techniques are described, for example, in Sambrook *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989. PCR cloning techniques are described, for
 20 example, in White, *Methods in Molecular Cloning*, volume 67, Humana Press, 1997.

MCH receptor probes and primers can be used to screen nucleic acid libraries containing, for example, genomic DNA or cDNA. Such libraries are commercially available, and can be produced using techniques such as those described in Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998.

25 Starting with a particular amino acid sequence and the known degeneracy of the genetic code, a large number of different encoding nucleic acid sequences can be obtained. The degeneracy of the genetic code arises because almost all amino acids are encoded by different combinations of nucleotide triplets or "codons". The translation of a particular codon into a particular amino acid is well
 30 known in the art (*see, e.g.*, Lewin *GENES IV*, p. 119, Oxford University Press, 1990). Amino acids are encoded by codons as follows:

- A=Ala=Alanine: codons GCA, GCC, GCG, GCU
 C=Cys=Cysteine: codons UGC, UGU
 D=Asp=Aspartic acid: codons GAC, GAU
 35 E=Glu=Glutamic acid: codons GAA, GAG

- F=Phe=Phenylalanine: codons UUC, UUU
 G=Gly=Glycine: codons GGA, GGC, GGG, GGU
 H=His=Histidine: codons CAC, CAU
 I=Ile=Isoleucine: codons AUA, AUC, AUU
 5 K=Lys=Lysine: codons AAA, AAG
 L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU
 M=Met=Methionine: codon AUG
 N=Asn=Asparagine: codons AAC, AAU
 P=Pro=Proline: codons CCA, CCC, CCG, CCU
 10 Q=Gln=Glutamine: codons CAA, CAG
 R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU
 S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU
 T=Thr=Threonine: codons ACA, ACC, ACG, ACU
 V=Val=Valine: codons GUA, GUC, GUG, GUU
 15 W=Trp=Tryptophan: codon UGG
 Y=Tyr=Tyrosine: codons UAC, UAU.

Nucleic acid having a desired sequence can be synthesized using chemical and biochemical techniques. Examples of chemical techniques are described in Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-
 20 1998, and Sambrook *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

Biochemical synthesis techniques involve the use of a nucleic acid template and appropriate enzymes such as DNA and/or RNA polymerases. Examples of such techniques include *in vitro* amplification techniques such as PCR and
 25 transcription based amplification, and *in vivo* nucleic acid replication. Examples of suitable techniques are provided by Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, Sambrook *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989, and Kacian *et al.*, U.S. Patent No. 5,480,784.

30 In different embodiments dog MCH receptor related nucleic acid comprises or consists of a unique nucleic acid region, or the complement thereof, at least 27 or at least 54 bases in length. Preferably, the dog MCH receptor related nucleic acid comprises or consists of the nucleic acid sequence of SEQ. ID. NO. 4.

MCH Receptor Probes

Detection probes for the dog MCH receptor preferably contain a unique dog nucleic acid region, or the complement thereof. Such probes can contain additional nucleic acid that may, or may not, be complementary to dog MCH receptor nucleic acid. Preferably, additional nucleic acid that is present has a particular purpose such as providing for increased specificity, being a reporter sequence, or being a capture sequence. However, additional nucleic acid need not have a particular purpose.

Probes for the MCH receptor can specifically hybridize to MCH receptor target nucleic acid under appropriate hybridization conditions (*i.e.*, distinguish target nucleic acid from one or more non-target nucleic acid molecules). A preferred non-target nucleic acid is either nucleic acid encoding for the human MCH receptor or the complement thereof. Hybridization occurs through complementary nucleotide bases present on the probe and MCH receptor nucleic acid. Hybridization conditions determine whether two molecules have sufficiently strong interactions with each other to form a stable hybrid.

Probes are composed of nucleic acids or derivatives thereof such as modified nucleic acid and peptide nucleic acid. Modified nucleic acid includes nucleic acid with one or more altered sugar groups, altered internucleotide linkages, and/or altered nucleotide purine or pyrimidine bases. References describing modified nucleic acid include International Publication No. WO 98/02582, U.S. Patent No. 5,859,221 and U.S. Patent No. 5,852,188, each of which are hereby incorporated by reference herein.

The degree of interaction between two molecules that hybridize together is reflected by the T_m of the produced hybrid. The higher the T_m the stronger the interactions and the more stable the hybrid. T_m is effected by numerous factors well known in the art such as the degree of complementarity, the type of complementary bases present (*e.g.*, A-T hybridization versus G-C hybridization), the structure of the nucleic acid backbones, and solution components. *E.g.*, Sambrook *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

Stable hybrids are formed when the T_m of a hybrid is greater than the temperature employed under a particular set of hybridization assay conditions. The degree of specificity of a probe can be varied by adjusting the hybridization stringency conditions. Detecting probe hybridization is facilitated through the use of a detectable

label. Examples of detectable labels include luminescent, enzymatic, and radioactive labels.

Examples of stringency conditions are provided in Sambrook *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989. An example of high stringency conditions is as follows: Prehybridization of filters containing DNA is carried out for 2 hours to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hours at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10⁶ cpm of ³²P-labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 minutes before autoradiography. Other procedures using conditions of high stringency would include, for example, either a hybridization step carried out in 5X SSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Recombinant Expression

MCH receptor related polypeptides can be expressed from recombinant nucleic acid in a suitable host or in a test tube using a translation system. Recombinantly expressed MCH receptor polypeptides are preferably used in assays to screen for compounds that bind to the MCH receptor and modulate the activity of the receptor.

Preferably, expression is achieved in a host cell using an expression vector. An expression vector contains recombinant nucleic acid encoding for a desired polypeptide along with regulatory elements for proper transcription and processing. The regulatory elements that may be present include those naturally associated with the recombinant nucleic acid and exogenous regulatory elements not naturally associated with the recombinant nucleic acid. Exogenous regulatory elements such as an exogenous promoter can be useful for expressing recombinant nucleic acid in a particular host.

Generally, the regulatory elements that are present include a transcriptional promoter, a ribosome binding site, a terminator, and an optionally present operator. Another preferred element is a polyadenylation signal providing for processing in eukaryotic cells. Preferably, an expression vector also contains an

origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high copy number. Examples of expression vectors are cloning vectors, modified cloning vectors, specifically designed plasmids and viruses.

5 Expression vectors that can be used to provide suitable levels of polypeptide expression in different hosts are well known in the art. Mammalian expression vectors well known in the art include pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt
10 (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), pCI-neo (Promega) and .lambda.ZD35 (ATCC 37565). Bacterial expression vectors well known in the art include pET11a (Novagen), lambda gt11 (Invitrogen), pcDNAII (Invitrogen), and pKK223-3 (Pharmacia). Fungal cell expression vectors well known in the art include pYES2 (Invitrogen), Pichia
15 expression vector (Invitrogen). Insect cell expression vectors well known in the art include Blue Bac III (Invitrogen).

Recombinant host cells may be prokaryotic or eukaryotic. Examples of recombinant host cells include the following: bacteria such as *E. coli*; fungal cells such as yeast; mammalian cells such as human, bovine, porcine, monkey and rodent;
20 and insect cells such as *Drosophila* and silkworm derived cell lines. Commercially available mammalian cell lines include L cells L-M(TK.sup.-) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC
25 CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

Expression vectors may be introduced into host cells using standard techniques. Examples of such techniques include transformation, transfection, lipofection, protoplast fusion, and electroporation.

30 MCH receptor nucleic acid can be expressed in a cell without the use of an expression vector employing, for example, synthetic mRNA or native mRNA. Additionally, mRNA can be translated in various cell-free systems such as wheat germ extracts and reticulocyte extracts, as well as in cell based systems, such as frog oocytes. Introduction of mRNA into cell based systems can be achieved, for example,
35 by microinjection.

EXAMPLES

Examples are provided below to further illustrate different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

5

Example 1: Cloning of a Dog MCH Receptor

A complete coding sequence for a dog MCH receptor was obtained by RT-PCR and hybridization screening of a cDNA library (constructed in the mammalian expression vector pcDNA 3.1 (+); Invitrogen) prepared from dog
 10 hypothalamus poly (A)+ mRNA. A forward (sense) PCR primer of SEQ. ID. NO. 17 (ATG GAC CTG) derived from a human MCH receptor sequence was used to amplify the dog MCH receptor. Thus, the resulting dog MCH receptor contains 6 amino acids (SEQ. ID. NO. 18: DLEASL) adjacent to the N-terminal methionine that may be similar or identical to those amino acids that correspond to the naturally
 15 occurring dog MCH receptor. The N terminal methionine (ATG) would be identical in both dog and human MCH receptors. The remaining 353 amino acids (amino acid 8 to 353) were from a dog MCH receptor.

The dog MCH receptor protein sequence is highly related to the human and rat MCH receptor of SEQ. ID. NOs. 2 and 3. The percent protein sequence
 20 identity to the human and rat MCH receptors is 97.5% and 94.6%, respectively.

The amino acid and encoding nucleotide sequences for the dog MCH receptor is as follows:

Dog MCH Receptor Amino Acid Sequence: SEQ. ID. NO. 1

25 MDLEASLLPPGPNASNTSEGPLDNLTSAGPPRRTGNVSYINIIMPSVFGTICLLGII
 GNSTVIFAVVKKSKLHWCSNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVW
 HFGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSVATLV
 ICLLWALSFISITPVWLYARLIPFPGGTVGCGIRLPNPDTDLYWFTLYQFFLAFA
 LPFVVITAAYVRILQRMMSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY
 30 YVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLSV
 KPAAQGQLRAVSNAQTADEERTESKGT

Dog MCH Receptor Encoding Nucleotide Sequence (including stop codon):SEQ. ID. NO. 4

ATGGACCTGGAAGCCTCGCTGCTGCCCCCGGCCCCAACGCCAGCAACAC
 CTCGGAGGGCCCGGACAACCTCACCTCTGCCGGGGCCACCTCGTCGCACAG
 5 GGAATGTCTCCTACATCAACATCATCATGCCTTCCGTGTTTCGGCACCATCT
 GCCTGCTGGGTATCATCGGGAACCTCCACAGTCATCTTCGCGGTGGTGAAG
 AAGTCCAAACTGCACTGGTGCAGCAATGTCCCCGACATCTTTATCATCAA
 CCTCTCGGTGGTAGACCTCCTCTTTCTCCTGGGGCATGCCCTTCATGATCCA
 CCAGCTCATGGGCAATGGTGTTTGGCATTTTGGAGAGACCATGTGCACAC
 10 TCATCACGGCCATGGACGCCAACAGTCAATTCACCAGCACCTACATCCTG
 ACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATCTCCTCC
 ACCAAGTTCCGGAAGCCCTCTGTGGCCACCCTGGTGATCTGCCTCCTATGG
 GCCCTCTCATTCATCAGCATCACCCCCGTGTGGCTCTACGCTAGGCTTATC
 CCCTTCCCAGGGGGGCACAGTGGGCTGTGGCATCCGCCTGCCCAACCCAGA
 15 CACTGACCTTTACTGGTTCACCCTGTACCAGTTCTTCCTGGCCTTTGCCCTG
 CCCTTCGTGGTCATCACAGCCGCGTATGTGAGGATCCTGCAGCGCATGAT
 GTCCTCGGTAGCCCCTGCCTCTCAACGCAGCATCCGGCTGCGGACAAAGA
 GGGTGACTCGCACGGCCATTGCCATCTGCCTGGTCTTCTTCGTGTGCTGGG
 CTCCCTACTATGTGCTACAGTTGACCCAGTTGTCCATCAGCCGCCCCGACAC
 20 TCACCTTTGTCTACCTGTACAACGCAGCCATCAGCTTGGGCTATGCCAACA
 GCTGCCTAAACCCCTTTGTGTACATCGTGCTCTGTGAGACATTCCGCAAGC
 GCTTGGTCCTGTCGGTGAAGCCTGCCGCCCAGGGGCAGCTTCGAGCCGTC
 AGCAATGCTCAGACAGCTGATGAGGAGAGGACAGAAAGCAAAGGCACCT
 GA

25

Example 2: Expression of Dog MCH Receptor

Measurement of MCH receptor expression in the aequorin-expressing
 stable reporter cell line 293-AEQ17 (Button *et al.*, 1993, *Cell Calcium*, 14, 663-671)
 was performed using a Luminoskan RT luminometer (Labsystems Inc., Gaithersburg,
 30 MD) controlled by custom software written for a Macintosh PowerPC 6100. 293-
 AEQ17 cells (8×10^5 cells plated 18 hours before transfection in a T75 flask) were
 transfected with 22 μ g of dog MCH receptor plasmid DNA: 264 μ g lipofectamine.

Following approximately 40 hours of expression the apo-aequorin in
 the cells was charged for 4 hours with coelenterazine (10 μ M) under reducing
 35 conditions (300 μ M reduced glutathione) in ECB buffer (140 mM NaCl, 20 mM KCl,

20 mM HEPES-NaOH [pH=7.4], 5 mM glucose, 1 mM MgCl₂, 1 mM CaCl₂, 0.1 mg/ml bovine serum albumin). The cells were harvested, washed once in ECB medium and resuspended to 500,000 cells/ml. 100 µl of cell suspension (corresponding to 5x10⁴ cells) was then injected into the test plate containing MCH, and the integrated light emission was recorded over 30 seconds, in 0.5 second units. 20 µL of lysis buffer (0.1% final Triton X-100 concentration) was then injected and the integrated light emission recorded over 10 seconds, in 0.5 second units.

The "fractional response" values for each well were calculated by taking the ratio of the integrated response to the initial challenge to the total integrated luminescence including the Triton X-100 lysis response. The EC₅₀ value for activation of the dog MCH receptor was ~ 30 nM.

Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.

SEQUENCE LISTING

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RECEPTOR

<130> 20668 PCT

<150> 60/219,669

<151> 2000-07-21

<160> 18

<170> FastSEQ for Windows Version 4.0

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<212> PRT

<213> Dog

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		20						25					30		
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Val	Val	Lys	Lys	Ser	Lys	Leu	His	Trp	Cys	Ser	Asn	Val	Pro	Asp	Ile
65				70					75					80	
Phe	Ile	Ile	Asn	Leu	Ser	Val	Val	Asp	Leu	Leu	Phe	Leu	Leu	Gly	Met
			85					90						95	
Pro	Phe	Met	Ile	His	Gln	Leu	Met	Gly	Asn	Gly	Val	Trp	His	Phe	Gly
		100						105					110		
Glu	Thr	Met	Cys	Thr	Leu	Ile	Thr	Ala	Met	Asp	Ala	Asn	Ser	Gln	Phe
		115					120					125			
Thr	Ser	Thr	Tyr	Ile	Leu	Thr	Ala	Met	Ala	Ile	Asp	Arg	Tyr	Leu	Ala
	130					135					140				
Thr	Val	His	Pro	Ile	Ser	Ser	Thr	Lys	Phe	Arg	Lys	Pro	Ser	Val	Ala
145				150					155						160
Thr	Leu	Val	Ile	Cys	Leu	Leu	Trp	Ala	Leu	Ser	Phe	Ile	Ser	Ile	Thr
			165					170						175	
Pro	Val	Trp	Leu	Tyr	Ala	Arg	Leu	Ile	Pro	Phe	Pro	Gly	Gly	Thr	Val
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Gly	Cys	Gly	Ile	Arg	Leu	Pro	Asn	Pro	Asp	Thr	Asp	Leu	Tyr	Trp	Phe
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Thr	Ala	Ala	Tyr	Val	Arg	Ile	Leu	Gln	Arg	Met	Met	Ser	Ser	Val	Ala
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Pro	Ala	Ser	Gln	Arg	Ser	Ile	Arg	Leu	Arg	Thr	Lys	Arg	Val	Thr	Arg
			245					250						255	
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Tyr	Val	Leu	Gln	Leu	Thr	Gln	Leu	Ser	Ile	Ser	Arg	Pro	Thr	Leu	Thr
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- 2 -

WO 02/08290

PCT/US01/22458

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 35      40      45
Thr Ile Cys Leu Leu Gly Ile Val Gly Asn Ser Thr Val Ile Phe Ala
 50      55      60
Val Val Lys Lys Ser Lys Leu His Trp Cys Ser Asn Val Pro Asp Ile
 65      70      75      80
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 85      90      95
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 100      105      110
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 165      170      175
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1

5

WHAT IS CLAIMED IS:

1. A purified polypeptide comprising a unique amino acid region of SEQ. ID. NO. 1 that is at least 9 contiguous amino acids in length, wherein said
5 unique region is not present in SEQ. ID. NO. 2 or SEQ. ID. NO. 3.
2. The polypeptide of claim 1, wherein said unique region comprises an amino acid sequence selected from the group consisting of:
SEQ. ID. NO. 7 LEASLLPPGP,
10 SEQ. ID. NO. 8 SEGPDNLTSAGP,
SEQ. ID. NO. 9 RRTGNVSYIN,
SEQ. ID. NO. 10 PFPGGTVGCG, and
SEQ. ID. NO. 11 ILQRMMSVA.
- 15 3. The polypeptide of claim 1, wherein said polypeptide comprises the amino acid sequence of SEQ. ID. NO. 1.
4. The polypeptide of claim 3, wherein said polypeptide consists of the amino acid sequence of SEQ. ID. NO. 1.
20
5. A purified nucleic acid comprising a nucleotide sequence encoding for the polypeptide of any one of claims 1-4.
6. A purified nucleic acid comprising a unique nucleotide
25 sequence region of SEQ. ID. NO. 4, or the complement thereof, that is at least 18 contiguous nucleotides in length, wherein said unique region is not present in SEQ. ID. NO. 5, SEQ. ID. NO. 6, or the complement of SEQ. ID. NO. 5 or SEQ. ID. NO. 6.
7. The purified nucleic acid of claim 6, wherein said unique
30 region comprises a nucleotide sequence selected from the group consisting of:
SEQ. ID. NO. 12 CCTCGGAGGGCCCGGACAACC,
SEQ. ID. NO. 13 ACCTCTGCCGGGCCACCTCGT,
SEQ. ID. NO. 14 GCCCTTCGTGGTCATCACAGCCGCGTAT,
SEQ. ID. NO. 15 TGTCCCTCGGTAGCCCCTGCCTCTCAA, and
35 SEQ. ID. NO. 16 TTCGAGCCGTCAGCAATGCT.

8. The purified nucleic acid of claim 6, wherein said nucleic acid comprises the nucleotide sequence of SEQ. ID. NO. 3.

9. The purified nucleic acid of claim 8, wherein said nucleic acid
5 consists of the nucleotide sequence of SEQ. ID. NO. 3.

10. A nucleic acid comprising a recombinant nucleotide sequence encoding for a polypeptide comprising a unique amino acid region of SEQ. ID. NO. 1 that is at least 9 contiguous amino acids in length, wherein said unique region is not
10 present in SEQ. ID. NO. 2 or SEQ. ID. NO. 3.

11. The nucleic acid of claim 10, wherein said unique region comprises an amino acid sequence selected from the group consisting of:
SEQ. ID. NO. 7 LEASLLPPGP,
15 SEQ. ID. NO. 8 SEGPDNLTSAGP,
SEQ. ID. NO. 9 RRTGNVSYIN,
SEQ. ID. NO. 10 PFPGGTVGCG, and
SEQ. ID. NO. 11 ILQRMMSSVA.

20 12. The nucleic acid claim 10, wherein said unique amino acid region comprises the amino acid sequence of SEQ. ID. NO. 1.

13. The nucleic acid of claim 10, wherein unique amino acid region consists of the amino acid sequence of SEQ. ID. NO. 1.

25

14. The nucleic acid of any one of claims 10-13, wherein said nucleic acid is an expression vector.

15. A recombinant cell comprising the expression vector of claim
30 14.

16. A recombinant cell made by a process comprising the step of introducing into said cell the expression vector of claim 14.

17. A method of measuring the ability of a test compound to affect MCH receptor activity comprising the steps of:

- a) contacting a recombinant cell with said compound, wherein said recombinant cell comprises a recombinant nucleic acid expressing a functional MCH receptor that comprises a unique amino acid region of SEQ. ID. NO. 1 that is at least 9 contiguous amino acids in length, provided that said unique region is not present in SEQ. ID. NO. 2 or 3; and
- b) measuring MCH receptor activity.

18. The method of claim 17, wherein said MCH receptor consists of the sequence of SEQ. ID. NO. 1.

19. The method of claim 18, wherein said contacting further comprises the use of a MCH agonist to stimulate MCH receptor activity, and measuring the ability of said compound to modulate MCH receptor activity.

20. A method of preparing a MCH receptor polypeptide comprising the step of growing the recombinant cell of claim 14 under conditions wherein said polypeptide is expressed from said expression vector.

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	1				50
huMCH1R	MDLEASLLPT	GPNASNTSDG	PDNLTSAGSP	PRTGSISYIN	IIMPSVFGTI
dogMCH1R	MDLEASLLPP	GPNASNTSEG	PDNLTSAGPP	RRTGNVSYIN	IIMPSVFGTI
ratMCH1R	MDLQTSLLST	GPNASNISDG	QDNLTLPGSP	PRTGSVSYIN	IIMPSVFGTI
	51				100
huMCH1R	CLLGIIGNST	VIFAVVKKSK	LHWCNNVPDI	FIINLSVVDL	LFLLGMPFMI
dogMCH1R	CLLGIIGNST	VIFAVVKKSK	LHWCSNVPDI	FIINLSVVDL	LFLLGMPFMI
ratMCH1R	CLLGIVGNST	VIFAVVKKSK	LHWCSNVPDI	FIINLSVVDL	LFLLGMPFMI
	101				150
huMCH1R	HQLMGNGVWH	FGETMCTLIT	AMDANSQFTS	TYILTAMAID	RYLATVHPIS
dogMCH1R	HQLMGNGVWH	FGETMCTLIT	AMDANSQFTS	TYILTAMAID	RYLATVHPIS
ratMCH1R	HQLMGNGVWH	FGETMCTLIT	AMDANSQFTS	TYILTAMTID	RYLATVHPIS
	151				200
huMCH1R	STKFRKPSVA	TLVICLLWAL	SFISITPVWL	YARLIPFPGG	AVGCGIRLPN
dogMCH1R	STKFRKPSVA	TLVICLLWAL	SFISITPVWL	YARLIPFPGG	TVGCGIRLPN
ratMCH1R	STKFRKPSMA	TLVICLLWAL	SFISITPVWL	YARLIPFPGG	AVGCGIRLPN
	201				250
huMCH1R	PDTDLYWFTL	YQFFLAFAFP	FVVITAAYVR	ILQRMTSSVA	PASQRSIRLR
dogMCH1R	PDTDLYWFTL	YQFFLAFAFP	FVVITAAYVR	ILQRMMSSVA	PASQRSIRLR
ratMCH1R	PDTDLYWFTL	YQFFLAFAFP	FVVITAAYVK	ILQRMTSSVA	PASQRSIRLR
	251				300
huMCH1R	TKRVTRTAIA	ICLVFFVCWA	PYYVLQLTQL	SISRPTLTFV	YLYNAAISLG
dogMCH1R	TKRVTRTAIA	ICLVFFVCWA	PYYVLQLTQL	SISRPTLTFV	YLYNAAISLG
ratMCH1R	TKRVTRTAIA	ICLVFFVCWA	PYYVLQLTQL	SISRPTLTFV	YLYNAAISLG
	301				350
huMCH1R	YANSCLNPFV	YIVLCETFRK	RLVLSVKPAA	QGQLRAVSNA	QTADEERTES
dogMCH1R	YANSCLNPFV	YIVLCETFRK	RLVLSVKPAA	QGQLRAVSNA	QTADEERTES
ratMCH1R	YANSCLNPFV	YIVLCETFRK	RLVLSVKPAA	QGQLRTVSNA	QTADEERTES
	351				
huMCH1R	KGT				
dogMCH1R	KGT				
ratMCH1R	KGT				

Fig. 1

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	1				50
huMCH1R	ATGGACCTGG	AAGCCTCGCT	GCTGCCCCACT	GGTCCCAACG	CCAGCAACAC
dogMCH1R	ATGGACCTGG	AAGCCTCGCT	GCTGCCCCCC	GGCCCCAACG	CCAGCAACAC
ratMCH1R	ATGGATCTGC	AAACCTCGTT	GCTGTCCACT	GGCCCCAATG	CCAGCAACAT
	51				100
huMCH1R	CTCTGATGGC	CCCGATAACC	TCACTTCGGC	AGGATCACCT	CCTCGCACGG
dogMCH1R	CTCGGAGGGC	CCGGACAACC	TCACCTCTGC	CGGGCCACCT	CGTCGCACAG
ratMCH1R	CTCCGATGGC	CAGGATAATC	TCACATTGCC	GGGGTCACCT	CCTCGCACAG
	101				150
huMCH1R	GGAGCATCTC	CTACATCAAC	ATCATCATGC	CTTCGGTGTT	CGGCACCATC
dogMCH1R	GGAATGTCTC	CTACATCAAC	ATCATCATGC	CTTCCGTGTT	CGGCACCATC
ratMCH1R	GGAGTGTCTC	CTACATCAAC	ATCATTATGC	CTTCCGTGTT	TGGTACCATC
	151				200
huMCH1R	TGCCTCCTGG	GCATCATCGG	GAACTCCACG	GTCATCTTCG	CGGTCGTGAA
dogMCH1R	TGCCTGCTGG	GTATCATCGG	GAACTCCACA	GTCATCTTCG	CGGTGGTGAA
ratMCH1R	TGTCTCCTGG	GCATCGTGCG	AAACTCCACG	GTCATCTTTG	CTGTGGTGAA
	201				250
huMCH1R	GAAGTCCAAG	CTGCACTGGT	GCAACAACGT	CCCCGACATC	TTCATCATCA
dogMCH1R	GAAGTCCAAA	CTGCACTGGT	GCAGCAATGT	CCCCGACATC	TTTATCATCA
ratMCH1R	GAAGTCCAAG	CTACACTGGT	GCAGCAACGT	CCCCGACATC	TTCATCATCA
	251				300
huMCH1R	ACCTCTCGGT	AGTAGATCTC	CTCTTTCTCC	TGGGCATGCC	CTTCATGATC
dogMCH1R	ACCTCTCGGT	GGTAGACCTC	CTCTTTCTCC	TGGGCATGCC	CTTCATGATC
ratMCH1R	ACCTCTCTGT	GGTGGATCTG	CTCTTCCTGC	TGGGCATGCC	TTTCATGATC
	301				350
huMCH1R	CACCAGCTCA	TGGGCAATGG	GGTGTGGCAC	TTTGGGGAGA	CCATGTGCAC
dogMCH1R	CACCAGCTCA	TGGGCAATGG	TGTTTGGCAT	TTTGGAGAGA	CCATGTGCAC
ratMCH1R	CACCAGCTCA	TGGGGAACGG	CGTCTGGCAC	TTTGGGGAAA	CCATGTGCAC
	351				400
huMCH1R	CCTCATCACG	GCCATGGATG	CCAATAGTCA	GTTCAACCAGC	ACCTACATCC
dogMCH1R	ACTCATCACG	GCCATGGACG	CCAACAGTCA	ATTCACCAGC	ACCTACATCC
ratMCH1R	CCTCATCACA	GCCATGGACG	CCAACAGTCA	GTTCACTAGC	ACCTACATCC
	401				450
huMCH1R	TGACCGCCAT	GGCCATTGAC	CGCTACCTGG	CCACTGTCCA	CCCCATCTCT
dogMCH1R	TGACCGCCAT	GGCCATTGAC	CGCTACCTGG	CCACTGTCCA	CCCCATCTCC
ratMCH1R	TGACTGCCAT	GACCATTGAC	CGCTACTTGG	CCACCGTCCA	CCCCATCTCC
	451				500
huMCH1R	TCCACGAAGT	TCCGGAAGCC	CTCTGTGGCC	ACCCTGGTGA	TCTGCCTCCT
dogMCH1R	TCCACCAAGT	TCCGGAAGCC	CTCTGTGGCC	ACCCTGGTGA	TCTGCCTCCT
ratMCH1R	TCCACCAAGT	TCCGGAAGCC	CTCCATGGCC	ACCCTGGTGA	TCTGCCTCCT

Fig. 2A

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	501				550
huMCH1R	GTGGGCCCTC	TCCTTCATCA	GCATCACCCC	TGTGTGGCTG	TATGCCAGAC
dogMCH1R	ATGGGCCCTC	TCATTTCATCA	GCATCACCCC	CGTGTGGCTC	TACGCTAGGC
ratMCH1R	GTGGGCGCTC	TCCTTCATCA	GTATCACCCC	TGTGTGGCTC	TACGCCAGGC
	551				600
huMCH1R	TCATCCCCTT	CCCAGGAGGT	GCAGTGGGCT	GCGGCATACG	CCTGCCCAAC
dogMCH1R	TTATCCCCTT	CCCAGGGGGC	ACAGTGGGCT	GTGGCATCCG	CCTGCCCAAC
ratMCH1R	TCATTCCCTT	CCCAGGGGGT	GCTGTGGGCT	GTGGCATCCG	CCTGCCAAAC
	601				650
huMCH1R	CCAGACACTG	ACCTCTACTG	GTTCAACCCTG	TACCAGTTTT	TCCTGGCCTT
dogMCH1R	CCAGACACTG	ACCTTTACTG	GTTCAACCCTG	TACCAGTTCT	TCCTGGCCTT
ratMCH1R	CCGGACACTG	ACCTCTACTG	GTTCACTCTG	TACCAGTTTT	TCCTGGCCTT
	651				700
huMCH1R	TGCCCTGCCT	TTTGTGGTCA	TCACAGCCGC	ATACGTGAGG	ATCCTGCAGC
dogMCH1R	TGCCCTGCCC	TTCGTGGTCA	TCACAGCCGC	GTATGTGAGG	ATCCTGCAGC
ratMCH1R	TGCCCTTCCG	TTTGTGGTCA	TTACCGCCGC	ATACGTGAAA	ATACTACAGC
	701				750
huMCH1R	GCATGACGTC	CTCAGTGGCC	CCCGCCTCCC	AGCGCAGCAT	CCGGCTGCGG
dogMCH1R	GCATGATGTC	CTCGGTAGCC	CCTGCCTCTC	AACGCAGCAT	CCGGCTGCGG
ratMCH1R	GCATGACGTC	TTCGGTGGCC	CCAGCCTCCC	AACGCAGCAT	CCGGCTTCGG
	751				800
huMCH1R	ACAAAGAGGG	TGACCCGCAC	AGCCATCGCC	ATCTGTCTGG	TCTTCTTTGT
dogMCH1R	ACAAAGAGGG	TGACTCGCAC	GGCCATTGCC	ATCTGCCTGG	TCTTCTTCGT
ratMCH1R	ACAAAGAGGG	TGACCCGCAC	GGCCATTGCC	ATCTGTCTGG	TCTTCTTTGT
	801				850
huMCH1R	GTGCTGGGCA	CCCTACTATG	TGCTACAGCT	GACCCAGTTG	TCCATCAGCC
dogMCH1R	GTGCTGGGCT	CCCTACTATG	TGCTACAGTT	GACCCAGTTG	TCCATCAGCC
ratMCH1R	GTGCTGGGCA	CCCTACTATG	TGCTGCAGCT	GACCCAGCTG	TCCATCAGCC
	851				900
huMCH1R	GCCCGACCCT	CACCTTTGTC	TACTTATACA	ATGCGGCCAT	CAGCTTGGGC
dogMCH1R	GCCCGACACT	CACCTTTGTC	TACCTGTACA	ACGCAGCCAT	CAGCTTGGGC
ratMCH1R	GCCCGACCCT	CACGTTTGTC	TACTTGTACA	ACGCGGCCAT	CAGCTTGGGC
	901				950
huMCH1R	TATGCCAACA	GCTGCCTCAA	CCCCTTTGTG	TACATCGTGC	TCTGTGAGAC
dogMCH1R	TATGCCAACA	GCTGCCTAAA	CCCCTTTGTG	TACATCGTGC	TCTGTGAGAC
ratMCH1R	TATGCTAACA	GCTGCCTGAA	CCCCTTTGTG	TACATAGTGC	TCTGTGAGAC
	951				1000
huMCH1R	GTTCCGCAAA	CGCTTGGTCC	TGTCGGTGAA	GCCTGCAGCC	CAGGGGCAGC
dogMCH1R	ATTCCGCAAG	CGCTTGGTCC	TGTCGGTGAA	GCCTGCCGCC	CAGGGGCAGC
ratMCH1R	CTTTCGAAAA	CGCTTGGTGT	TGTCAGTGAA	GCCTGCAGCC	CAGGGGCAGC

Fig. 2B

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	1001		1050
huMCH1R	TTCGCGCTGT CAGCAACGCT CAGACGGCTG ACGAGGAGAG GACAGAAAGC		
dogMCH1R	TTCGAGCCGT CAGCAATGCT CAGACAGCTG ATGAGGAGAG GACAGAAAGC		
ratMCH1R	TCCGCACGGT CAGCAACGCT CAGACAGCTG ATGAGGAGAG GACAGAAAGC		
	1051	1062	
huMCH1R	AAAGGCACCT	GA	
dogMCH1R	AAAGGCACCT	GA	
ratMCH1R	AAAGGCACCT	GA	

Fig. 2C